The bond energies (Vedeneev *et al.*, 1966) in kcal mol^{-1} are given beneath the bonds affected, the slash indicating the bond about to be broken. The enthalpy of reaction (ΔH_R) is also given. It is noted that the only endothermic process is that shown by Scheme III. However, when bond energy errors are considered, the energy input could be (4 ± 14) kcal mol^{-1}. The observed activation energies obtained in this paper are larger than the enthalpies of reaction of the three possible schemes. This is the expected result as the enthalpy of reaction is the minimum activation energy.

Morawetz and Otaki (1963) observed that Scheme III best explained the mechanism for the formation of amides in aqueous solution. In addition, they showed that the activation energy decreased with increasing chain length, as observed here for the solid state. However, the activation energies they obtained were approximately half those of the present study. The differences may be caused by the different acids employed for the amidification.

As long as adequate temperature control is used, quantitative yields of amides can be obtained. These results agree with those of Hunter (1941), who prepared long-chain amides of many acids, including phenylacetic acid, by pyrolysis in sealed tubes.

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Estimation of Thiabendazole in the Milligram and Submilligram Ranges

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A procedure is described for the estimation of thiabendazole suspensions of ~0.1–2.0 mg/ml concentration. The procedure depends on the extraction of a copper-thiabendazole-tetramethylguanidine complex by chloroform from an aqueous suspension at pH 12.0–12.5. Absorbance of the complex is measured at 350–400 nm, or the color may be compared with artificial permanent standards. In a modification that permits estimation of ~2 μg/ml, absorbance of the copper-thiabendazole-tetramethylguanidine complex in chloroform is measured at 311 nm.

Aqueous thiabendazole [2-(4′-thiazolyl)benzimidazole] (TBZ) suspensions of up to 1000 ppm are widely used as a soak for agricultural products to control diseases and spoilage caused by *Fusarium, Penicillium,* and other fungi. Methods available to determine the concentration of TBZ in the soak tanks were summarized by Holmes (1972). These included measurement of the absorbance of a dilute hydrochloric acid solution of TBZ at 302 nm, a titration using silver nitrate, and estimation by refractive index. Miller *et al.* (1971) proposed an indirect estimation by determining excess methylmercuric chloride from the reaction of a known amount of methylmercuric chloride with TBZ. The color of the methylmercuric dithizonate formed from the excess methylmercuric chloride and a known amount of dithizone was compared with artificial permanent color standards. Silver nitrate may be substituted for methylmercuric chloride in this reaction (Miller and Csonka, 1973). A test kit for TBZ from Lamotte Chemical Co. is now available using the official AOAC procedure (1965). However, a rapid tankside test is needed in some industries, such as the flower bulb industry in Washington State.

Miller *et al.* (1971) observed that the copper ion reacts with TBZ in alkaline suspension to form a deep green insoluble compound. Under proper conditions, a similar compound may be extracted rapidly by chloroform. The resulting chloroform solution may be measured spectrophotometrically or compared with artificial permanent color standards to estimate TBZ in the soaking tanks.

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MATERIALS AND METHODS

For the estimation of TBZ in bulb treating tanks, up to 20 ml of a carefully mixed sample of the tank suspension containing 2–12 mg of TBZ is placed in a separatory funnel. If the sample volume is <10 ml, water is added to make the volume to 10 ml. Two-tenths of a milliliter of practical grade tetramethylguanidine (TMG) is added, followed by 10.0 ml of chloroform. After the addition of 0.5 ml of 1% cupric chloride dihydrate, the funnel is shaken vigorously at once for 1 min. The layers are allowed to separate and the chloroform phase is passed through a small plug of cotton in the stem of the separatory funnel to remove suspended water. The absorbance of the chloroform phase may be measured spectrophotometrically in a cuvette of 1.00 cm light path at any selected wavelength from 350 to 400 nm and compared with the absorbance of 2.0–12.0 mg of TBZ carried through the procedure and measured at the same wavelength.

Comparison of the chloroform phase may be made with permanent color standards in similar tubes. The composition of the color standards representing 2.0–12.0 mg of TBZ as copper-TBZ-TMG complex in 10 ml of chloroform is given in Table I.

A modified procedure may be used to determine ~2 μg/ml of TBZ. The solution or suspension of TBZ is made alkaline with 0.2 ml of TMG, and 5.00 ml of chloroform is added, followed by 0.1 ml of 1% cupric chloride dihydrate. After extraction and filtration of the chloroform phase

Table I. Composition of Permanent Standards for Testing Thiabendazole Concentrations. The Indicated Volumes of the Solutions for Each Standard Are Combined in a 18-mm Outside Diameter Test Tube. The Tube Is Closed with a Melted Glass Seal

Reagent	g/100 ml of solution	Thiabendazole equivalent of standard, mg					
		12.00	10.00	8.00	6.00	4.00	2.00[b]
H_2O		6.35 ml	7.00 ml	7.50 ml	7.88 ml	8.30 ml	8.70 ml
$Ni[NO_3]_2 \cdot 6H_2O$	37.50[a]	2.78	2.22	1.84	1.28	0.90	0.40
$K_2Cr_2O_7$	1.00	0.21	0.18	0.16	0.12	0.07	0.05

[a] Contained 1.0 ml of 16 *N* nitric acid. [b] Equivalent to 1200, 1000, 800, 600, 400, and 200 ppm with a 10.0-ml sample.

Table II. Thiabendazole in Suspensions Estimated by Different Methods Before and After Addition of Contaminants

Before addition of contaminant, ppm				After addition of contaminant, ppm			
	Absorbance at			Absorbance at			
By weight	302 nm	375 nm	Visual	302 nm	375 nm	Visual	Contaminant added/200 ml of suspension
784	788	776	800	776	757	700	2.0 g of iris husk
878	853	875	900	874	932	800	3.0 g of iris husk
657	644	650	700	652	652	600	5.0 g of iris bulb
804	863	790	800	771	790	750	5.0 g of iris bulb
593	645	615	600	554	545	550	5.0 g of iris bulb
233	191	207	200	222	214	200	1.5 g of soil

Table III. Amines in Groups Determined by Absorbance from the Reaction with 5 mg of Thiabendazole

Group I, absorbance >1.000	Group II, absorbance 0.500–1.000	Group III, absorbance 0.200–0.500	Group IV, absorbance <0.200
1-Dimethylamino-2-propanol	3-Amino-1-propanol	2-Aminobutane	1-Amino-2-propanol
2-Dimethylamino-2-methyl-1-propanol	Dimethylaminoethanol	Diethylamine	Ethanolamine
3-Dimethylamino-1-propanol	Ethylamine	Dimethylaminocyclohexane	2-Amino-2-methylpropanol
	N-Methylpyrollidone	3-Picoline	Diethanolamine
	Piperazine	Tetrapropylammonium-hydroxide	(Methylamino)ethanol
	Piperidine	Tri-*N*-octylamine	Morpholine
	Tetramethylguanidine		Methylethanolamine
			Methyldiethanolamine
			Triethanolamine
			Triethylenetetramine
			Tetraethylenepentamine
			Dimethylaniline

through a cotton plug, the absorbance is determined at 311 nm and compared with known amounts of TBZ carried through the same procedure. A peak in the absorbance of the copper-TBZ-TMG in chloroform occurred at 310–312 nm, a wavelength approximately 10 nm longer than the peak for TBZ in dilute HCl or in chloroform.

Suspensions of formulated TBZ (Mertect) were prepared comparable to those used in the bulb industry. The concentration of TBZ was determined from the weight of the formulation used by the proposed procedure using the permanent standards and by spectroscopy at 375 nm and spectroscopy of the TBZ in 0.1 *N* HCl at 302 nm. Materials sometimes added to the bulb-treating tanks, such as formalin and aldrin wettable powder, and material inadvertently present such as soil, crushed bulbs, and bulb husks were added and the analyses repeated 2 days later. Typical results are shown in Table II before and after addition of the indicated amounts of contaminant to 200 ml of TBZ suspension.

RESULTS

The data in Table II indicate that both the results from absorbance measurement at 375 nm or by visual observation using the permanent standards will yield satisfactory results for testing bulb soaking suspensions. The materials likely to contaminate suspensions of TBZ as used in the bulb industry, such as crushed bulbs, bulb husks, and sandy or loamy soil, did not interfere in the procedure. Intractible emulsions were caused by 1 g of peat soil/100 ml of TBZ suspension. Minor emulsions may be resolved by filtration through phase separating paper. Other materials sometimes added to bulb-treating tanks, such as aldrin wettable powder or formalin, had no effect. Numerous additional samples confirm these observations.

The method for ~2 μg/ml may be used to concentrate small amounts of TBZ from very dilute solutions or suspensions. When 10 μg of TBZ was extracted from 10 ml of aqueous suspension using TMG, copper, and 5.0 ml of chloroform, the absorbance was some 1.5 times greater than the same amount of TBZ measured at 302 nm in 10.0 ml of 0.1 *N* HCl.

DISCUSSION

For the reaction to be quantitative and reproducible, the pH of the reaction medium must be 12.0–12.5. This pH was attained in the described procedure, even if as much as 0.5 ml of *N* HCl was added. Some color was formed and extracted at pH as low as 10.5. Two grams of sodium chloride and 0.2 g of trisodium phosphate, sodium citrate, or sodium acetate did not interfere. Numerous solvents were found that would extract the colored complex. These included methylene chloride, methylchloroform, amyl alcohol, amyl acetate, carbon tetrachloride, and ethyl acetate (with some decomposition). The first three appeared to be satisfactory but offered no advantage over chloroform, and generally the amount of colored material extracted was less.

The structure of the compound extracted into chloroform was not eludicated. However, the Cu:TBZ molar ratio was 1:2. The copper was determined by extraction of

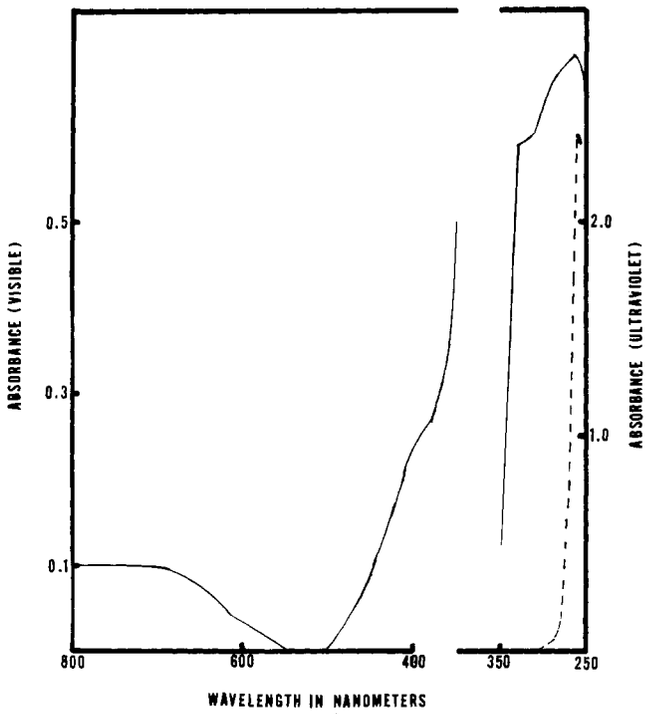

Figure 1. Absorption spectra of the copper–thiabendazole–tetramethylguanidine complex (——) and copper–tetramethylguanidine complex (- - -) in chloroform.

the chloroform solution of the compound with N HCl and analysis of the copper present by the procedure of Williams and Morgan (1954). The molar amount of TMG in the compound was not determined. The TMG serves a dual function of pH adjustment as well as a component of the extracted compound. TMG partitions almost equally between the aqueous and chloroform phases. The volume of TMG represents a considerable excess of this reagent but when lesser (10–20%) amounts were used, the maximum color failed to develop and tended to fade on standing with the development of insoluble material.

Other amines substituted for TMG produced variable amounts of color extractable by chloroform (Table III). If 0.2 ml of the amine would not give a pH > 10, 0.2 ml of 0.4 N sodium hydroxide was added. The maximum extractable color was produced by 1-dimethylamino-2-propanol, 2-dimethylamino-2-methyl-1-propanol, and 3-dimethylamino-1-propanol. All contained a dimethylamino and an α- or β-hydroxyl in a three-carbon chain. Several types of amines were in the second group, including TMG. Group 3 amines produced less color, generally yellow, and group 4 produced little or no extractable color. TMG was chosen as the most suitable reagent because the color was stable, and small differences in volume of the sample, copper chloride solution, or TMG did not affect the color extracted. Since the test was designed as a tankside test, the sensitivity was adequate. The reaction can be carried out in two steps, with the TBZ extracted into chloroform from an alkaline suspension, followed by reaction in a second separatory funnel with copper ion. No advantage was observed. The compound is not stable to acid, but the chloroform may be shaken with an equal volume of water once without diminution of the color.

Several salts of copper, such as the chloride, nitrate, sulfate, gluconate, and alkaline tartarate, were found to be satisfactory if used in equimolar amounts. Fifty milligrams of tetrasodium ethylenediaminetetraacetate interfered. Miller *et al.* (1973) reported that both nickel and cobalt give colored compounds with TBZ. However, neither produced a colored compound with TMG that could be extracted from the alkaline aqueous phase into the chloroform phase. Benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] interfered in the procedure. The absorbance of the greenish yellow color measured at 375 nm was ~35% as much as from the same weight of TBZ. An equal amount of benzimidazole carried through the reaction did not produce measurable absorbance.

The absorbance of the deep green color of the copper–TBZ–TMG complex could be measured at any selected wavelength from 350 to 400 nm. The absorbance of the copper–TBZ–TMG complex in chloroform increased slowly from 530 to 360 nm and then rapidly to a peak at 335 nm. At the other end of the spectrum, the absorbance increased slowly from 530 nm to a broad peak at 700 nm, with considerable absorption from 600 to over 800 nm. Thus, the green color observed appeared to be a blend of the yellow and blue, rather than a peak in the green portion of the spectrum. The reagent blank in chloroform does not absorb appreciably above 315 nm. A graph of the absorption spectra of the copper–TBZ–TMG complex from 1.0 mg of TBZ determined using a Hitachi Perkin-Elmer spectrophotometer is presented in Figure 1. In the micro procedure for ~2 μg/ml of TBZ, the spectra were different between 270 and 330 nm. A peak was observed between 309 and 312 nm that is absent in the spectra of the larger amount of TBZ, as shown in Figure 1.

The chloroform extract from the sample plus TMG must be colorless, since extraneous color in the sample would make comparison with the artificial standards difficult, if not impossible. Blank runs should be made without copper ion to determine the applicability of the procedure to each system to be tested.

The accuracy of an individual test by visual observation using the permanent standards cannot be expected to be better than ±50 ppm. The means of the different methods of estimation before addition of contaminants (Table II) were within ±1%. After addition of the contaminants, the mean of the visual observation data was 6% lower than the data from 302 nm readings, but the mean of the analyses at 375 nm was within 1% of the mean of the analyses at 302 nm.

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